REMARKS

Claims 1–4, 6, 8–17, and 19–24 are pending in the application. Claims 1–4, 6, and 8–17, and 19–24 stand rejected. Claims 1, 6, and 21 have been amended. No new matter has been introduced. Reconsideration and allowance of Claims 1–4, 6, 8–17, and 19–24 are respectfully requested.

The Rejection of Claims 1, 3–4, 6, 8, 10, 12, 15, and 19–21 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Lishanski, *Clinical Chemistry 46*(9):1464–1470 (2000), in View of Lau et al., *Science 294*:858–862 (2001), as Evidenced by Lau, Supplemental Information.

Claims 1, 3–4, 6, 8, 10, 12, 15, and 19–21 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Lishanski, *Clinical Chemistry 46*(9):1464–1470 (2000) ("Lishanski") in view of Lau et al., *Science 294*:858–862 (2001), and Supplemental Information ("Lau"). Applicant respectfully traverses this ground of rejection for at least the following reasons.

While not acquiescing to the Examiner's position, but in order to facilitate prosecution, Claims 1, from which Claims 2–4, 6, 8, 10, 12, 15, and 19–20 depend, and Claim 21, from which Claims 22–24 depend, have been amended. Claims 1 and 21 as amended now recite in part at step (a) and step (1), respectively, "an extension primer comprising a first portion having a length from 3 to 17 nucleotides selected to hybridize to a portion of the target microRNA molecule." Additionally, Claim 6 has been amended and now recites "wherein the first portion of the extension primer has a length in the range of from 6 to 17 nucleotides." Support for these amendments is found throughout the specification as filed; for example, at page 7, lines 24–26; Table 1 (listing extension primers with first portions up to, but that do not exceed 17 nucleotides and that were successfully employed to produce DNA molecules from microRNA target molecules); and Tables 2 6, 7, and 8.

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The cited references either alone or in combination fail to render the claimed invention unpatentable. *KSR* confirmed that the Graham Factor Analysis should be used in determining whether a claimed invention is obvious under Section 103(a). *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1739 (2007). This analysis includes assessing the rejected claims, the scope and content of the cited art, and the differences between the rejected claims and the cited art. *Id.* at 1734. As will be shown, a *prima facie* case of obviousness has not been established because: (1) the references taken together or separately, fail to teach every limitation of the claimed invention; (2) the hypothetical combination proposed by the Examiner does not result in the claimed invention; and (3) there is no motivation or expectation of success to combine the references to arrive at the claimed invention because, at minimum, the Examiner's proposed treatment of the prior art would render the method of Lau unsatisfactory for its intended purpose.

1. The Differences Between the Rejected Claims, as Amended, and the Cited Art

Lishanski

Lishanski is generally directed toward screening for single nucleotide polymorphisms (SNPs) in genomic DNA using a "branch migrations" analysis. The analysis is based on polymerase chain reaction amplification of the target genomic DNA sequence using two forward and two reverse primers. All primers contain sequences that specifically anneal to the sequence of the target DNA. The reverse primers have the same target specific sequence, but also contain different unique tail sequences (t1 or t2) that do not hybridize with the target DNA. The forward primers have the same target specific sequence, but are 5' end-labeled with either biotin or digoxigenin. After PCR amplification and heat denaturization, the single stranded amplicons form various structures, including doubly labeled four-stranded cruciform DNA structures. If the source DNA genomic target was heterozygotic for a SNP, sufficient cruciform structures will maintain their shape and emit a detectable signal upon ELISA or LOCI analysis. As noted by the Examiner, Lishanski also teaches an alternative approach in which the forward primer is a mix

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that includes an adapter primer with a target specific 3' domain and a 5' domain that is identical to a universal primer. After the initial rounds of PCR, the adapter primer generates enough amplicon to serve as a template for the labeled universal primers, which are also included in the mix. As illustrated in Exhibit I, Lishanski used primers to amplify the genomic DNA targets that contained target-specific sequences that ranged from at least 19 nucleotides to 25 nucleotides.

Nowhere does Lishanski teach or remotely suggest using an extension primer comprising a first portion from 3 to 17 nucleotides in length that hybridizes to a target microRNA. Further, Lishanski does not teach or suggest a reverse primer for amplifying a cDNA molecule comprising a sequence selected to hybridize to a portion of the cDNA molecule comprising a sequence complementary to the target microRNA, as presently recited in Claims 1 and 21.

Lau et al.

The Examiner admits that Lau does not teach the use of an extension primer comprising a first portion that hybridizes to a target microRNA. See Office Action mailed June 24, 2009, at page 11. It is further noted that Lau does not teach or suggest amplifying the first DNA molecule to produce amplified DNA molecules using the universal forward primer and a reverse primer, wherein the reverse primer is selected to specifically hybridize to a portion of the first DNA molecule that is complementary to the target microRNA molecule under defined hybridization conditions.

Rather, in sharp contrast to the claimed invention, which is directed to methods for quantitating specific target microRNAs, Lau is directed to the non-specific amplification of all microRNAs in a sample through the ligation of adaptor linkers for the purpose of cloning previously unidentified microRNA species. In particular, Lau describes the cloning of endogenous *C. elegans* miRNAs and the discovery of 55 previously unknown miRNAs in *C. elegans*. Lau, Abstract. Lau describes the construction of an amplified small RNA library by first ligating 3' RNA adaptor oligonucleotides to a pool of gel-purified 18–26 nucleotide small

RNAs from mixed-stage worms with T4 RNA ligase, gel purifying the ligated RNA, then ligating to a 5' adaptor oligonucleotide in a second T4 RNA ligase reaction, gel purifying the products from the second ligation followed by reverse transcription and PCR amplification of the linker-ligated products using DNA oligos corresponding to the adaptor sequences. The PCR products were submitted for sequencing. See Footnote 23 and Lau supplementary materials.

Nowhere does Lau teach or remotely suggest using an extension primer comprising a first portion from 3 to 17 nucleotides in length that hybridizes to the target microRNA. Further, Lau does not teach or suggest a reverse primer for amplifying a cDNA molecule comprising a sequence selected to hybridize to a portion of the cDNA molecule comprising a sequence complementary to the target microRNA, as presently recited in Claims 1 and 21.

2. The Differences Between the Rejected Claims and the Cited Art Are Not Obvious Differences

The Examiner suggests that the method of Lishanski could be applied to the detection of a variety of targets, including microRNA targets as taught by Lau. The Examiner concludes that one of ordinary skill in the art at the time the invention was made would have been motivated to have applied the format of tagged and tailed primers of Lishanski to the detection of microRNA sequences as taught by Lau to arrive at the claimed invention with a reasonable expectation of success. Applicant respectfully disagrees.

There is no apparent reason to modify the teachings of Lishanski with the teachings of Lau in the manner proposed by the Examiner. In the context of an obviousness rejection, the Supreme Court explained the importance of "identifying a reason" why a skilled artisan would be prompted to arrive at the presently claimed invention. *KSR*, 127 S.Ct. at 1741. The Court noted that there should be an "explicit" analysis regarding "whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *Id.*

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As a preliminary matter, because the combination of references fails to teach every limitation of the present claims, there can be no "apparent reason" to combine the references to arrive at the claimed invention. In this regard, neither reference teaches or suggests an extension primer comprising a first portion having a length from 3 to 17 nucleotides selected to hybridize to the target microRNA, as recited in the currently amended Claims 1 and 21.

Moreover, there is no reasonable expectation of success to modify Lau with the teachings of Lishanski in the manner proposed by the Examiner. Barad et al., *Genome Research 14*:2486–2494 (2004) at page 2487, first column, teaches that "the detection of microRNA expression has met with significant difficulties, mostly due to their small size, and the relative poor sensitivity and low comparative power of the current methods used for their detection." (See Barad (2004) submitted herewith in the Supplemental Information Disclosure Statement). Lau teaches that microRNAs are about 21- to 24- nucleotides in length. Lau, Abstract. The present specification starting at page 8, line 16, explains that the reverse primer used to amplify the cDNA should be long enough to have a sufficiently high melting temperature for the PCR, but not so long as to have extensive overlap with the extension primer. This minimization of overlap is intended to avoid the formation of primer dimers. The hypothetical combination suggested by the Examiner, wherein Lau is modified to possess the primers with a tag sequence and a portion specific to the target, as taught by Lishanski, would result in extensive primer overlap leading to high primer dimerization levels. High primer dimerization, in turn, would lead to elevated background and lower sensitivity in the quantitation of target in a sample. In this regard, as noted above and in Exhibit I, the target specific portions of the Lishanski primers ranged from 19 to 25. Therefore, many of these hypothetical extension primers would hybridize to the entire length of their target and, thus, would completely overlap with the reverse primer. Neither Lau nor Lishanski provide any guidance whatsoever to avoid problems, such as primer dimerization, that arise in the amplification of such short targets using target-specific primers. Therefore, the combination of

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references as proposed by the Examiner would not result in methods with the features and advantages of the present invention.

Further, there is no motivation to modify Lau to include primers with a tag sequence and target-specific sequence as taught by Lishanski as proposed by the Examiner, because to do so would render the teaching of Lau unsatisfactory for its intended purpose. Lau teaches the non-specific amplification of all microRNAs in a sample in order to recover the sequences, and profile the expression, of all the microRNAs present in the sample. See Lau, page 858, second column, and Lau page 862, no. 23, for a description of the method. In contrast, the hypothetical use in Lau of target specific primers would first require *a priori* knowledge of the target sequence, and second would limit the amplification to only that target, thus, causing Lau to lose the ability to create a profile of all microRNA's present in a sample.

In view of the foregoing, it is demonstrated that a *prima facie* case of obviousness has not been established because Lau and Lishanski, taken together or separately, fail to teach every limitation of Claims 1 and 21, as currently amended. Furthermore, it is demonstrated that a person of ordinary skill in the art would not modify the references to arrive at the present invention as recited in Claims 1 and 21, as currently amended. Claims 3–4, 6, 8, 10, 12, 15, and 19–20 depend from Claim 1. Therefore, removal of this ground of rejection is respectfully requested.

The Rejection of Claims 2, 13–14, 16–17, and 22 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Lishanski, in View of Lau et al., as Evidenced by Lau Supplemental Information, as Applied Above, in Further View of Braasch et al.

Claims 2, 13–14, and 16–17, which depend from Claim 1, and Claim 22, which depends from Claim 21, stand rejected under 35 U.S.C. § 103(a) as unpatentable over Lishanski, *Clinical Chemistry 46(9)*:1464–1470 (2000), in view of Lau et al., *Science 294*:858–862 (2001), as

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evidenced by Lau Supplemental Information, as applied above, in further view of Braasch et al., *Chemistry & Biology* 8:1–7 (2001). Applicant respectfully traverses this ground of rejection for at least the following reasons.

As described above with respect to Claims 1 and 21, Lishanski and Lau, either separately or together, fail to teach or suggest every element of the claimed invention. Braasch fails to remedy the deficiencies of Lishanski and Lau. Braasch merely teaches that locked nucleic acids are nucleic acid analogs that may be incorporated in DNA and RNA oligomers. Braasch does not teach or suggest an extension primer comprising a first portion having a length from 3 to 17 nucleotides that hybridizes to the target microRNA. Further, Braasch does not teach or suggest a reverse primer for amplifying a cDNA molecule comprising a sequence selected to hybridize to a portion of the cDNA molecule comprising a sequence complementary to the target microRNA, as recited in Claims 1 and 21.

Thus, for at least the reasons discussed above in connection with independent Claims 1 and 21, dependent Claims 2, 13–14, 16–17, and 22 are not obvious over the cited art. Applicant respectfully requests withdrawal of this ground of rejection.

The Rejection of Claims 9 and 11 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Lishanski, in View of Lau et al., as Evidenced by Lau Supplemental Information, as Applied Above, in Further View of Crollius et al. and Buck et al.

Claims 9 and 11 which depend from Claim 1, stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Lishanski, *Clinical Chemistry* 46(9):1464–1470 (2000) in view of Lau et al., *Science* 294:858–862 (2001), as evidenced by Lau Supplemental Information, as applied above, in further view of Crollius et al., *Nature Genetics* 25(2):235–238 (2000) and Buck et al., *Biotechniques* 27:528–536 (1999). In this rejection, the Examiner also makes reference to

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Spivack et al., U.S. Patent Publication No. 2003/0186288 ("Spivack"). Applicant respectfully traverses this ground of rejection for the following reasons.

As described above with respect to Claim 1, Lishanski and Lau, either separately or together, fail to teach or suggest every element of the claimed invention. Neither Crollius, Buck, nor Spivack, separately or together, remedy the deficiencies of Lishanski and Lau. The Examiner characterizes Crollius as teaching an extension primer comprising the nucleic acid sequence of SEQ ID NO:1 and wherein a universal forward primer consists of the nucleic acid sequence set forth in SEQ ID NO:13. The Examiner characterizes Buck as demonstrating the equivalence of primers selected by various different criteria for amplifying a target sequence. The Examiner characterizes Spivack as teaching universal RT primers with a unique 5' tag that initiate reverse transcription and provide a target for universal PCR primers. It is noted that Crollius, Buck, and Spivack, either alone or in combination, do not teach or suggest an extension primer comprising a first portion having a length from 3 to 17 nucleotides selected to hybridize to the target microRNA, or a reverse primer for amplifying a cDNA molecule comprising a sequence selected to hybridize to a portion of the cDNA molecule comprising a sequence complementary to the target microRNA, as recited in Claim 25, as amended.

Thus, for at least the reasons discussed above in connection with independent Claim 1, dependent Claims 9 and 11 are not obvious over the cited art. Applicant respectfully requests withdrawal of this ground of rejection.

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The Rejection of Claims 23–24 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Lishanski, *Clinical Chemistry* 46(9):1464–1470 (2000) in View of Lau et al., *Science 294*:858–862 (2001), as Evidenced by Lau Supplemental Information, as Applied Above, in Further View of U.S. Patent Publication No. 2003/0186288 (Spivack et al.)

Claims 23–24, which depend from Claim 21, stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Lishanski in view of Lau, in further view of U.S. Patent Publication No. 2003/0186288 ("Spivack"). Applicant respectfully traverses this ground of rejection for at least the following reasons.

As described above with respect to Claim 21, Lishanski and Lau, either separately or together, fail to teach or suggest every element of the claimed invention. Further, no motivation exists to combine these references, and there is no reasonable expectation of success to achieve the claimed invention with respect to such a hypothetical combination. The teachings of Spivack fail to remedy the deficiencies of Lishanski and Lau.

The Examiner characterizes Spivack as teaching the amount of amplified DNA molecules being measured by fluorescence-based quantitative PCR, including an embodiment wherein the amount of amplified DNA molecules are measured using SYBR green dye. It is noted that there is no teaching or remote suggestion in Spivack with regard to the use of an extension primer comprising a first portion having a length from 3 to 17 nucleotides that hybridizes specifically to a target microRNA, as claimed. Rather, Spivack discloses the use of an RT-PCR strategy that detects and primes mRNA with a primer that hybridizes to the polyA tail of the all mRNA in a sample. See, for example, paragraph [0103] of Spivack, which states, "The methods of the present invention can be used to analyze gene expression from any material in which genes are expressed to generate mRNA molecules having poly-A tails." As described in paragraph [0057] of Spivack, the RT primers used to perform reverse transcription comprise (a) a 3' anchor sequence; (b) a poly T midsection to anneal to the polyA tail of the mRNA; and (c) a 5' tag.

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Spivack et al. does not disclose or suggest a method using an extension primer that specifically hybridizes to a target microRNA as claimed, because, as known by those of skill in the art, a mature microRNA does not contain a polyA tail. Furthermore, as described by the Examiner, the 3' anchor sequence covers all possible combinations of the coding 3' end of the mRNA transcripts. This merely permits the anchoring of the universal at the 5' end of the poly-A tail for all mRNA transcripts in the sample, thus maintaining the universality of the primer.

The combination proposed by the Examiner would not result in the claimed invention because there is no ability in any of the cited references to specifically prime and quantitatively measure a particular target microRNA. Rather, in sharp contrast to the claimed invention, which is directed to methods for quantitating specific target microRNAs, the teachings of Lau and Spivack are both directed to the non-specific priming and/or amplification of all microRNAs in a sample either through the ligation of adaptor linkers (Lau), or through the use of universal oligo-dT priming of all polyA+ mRNA (Spivack). Thus, no combination of the cited references renders the claimed invention obvious. See M.P.E.P. § 2143.02 and *KSR*, 127 S.Ct. at 1741.

As noted above, Spivack describes the use of an RT-PCR strategy that detects mRNA with a primer that hybridizes to the polyA tail of the mRNA. Contrary to the Examiner's contention, Spivack does not teach or suggest a method comprising a primer that specifically hybridizes to a target microRNA, because, as known by those of skill in the art, a mature microRNA does not contain a polyA tail. The 3' anchor covers all possible combinations of the coding region, and thus is still used for universal priming. Therefore, there is no apparent reason why one of skill in the art would modify the teachings of Lishanski or Lau to include a region to hybridize to a polyA tail when the target microRNA is known to lack a polyA tail. In fact, Spivack actually teaches directly away from the claimed invention with the teaching that the use of target-specific extension primers are undesirable due to the requirement of very specific annealing conditions, the fact that new reverse transcription primers would be required for each

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transcript target to be analyzed, and the inefficient use of RNA in target-specific analysis (page 2, paragraphs [0010] – [0013]).

Therefore, it is demonstrated that a *prima facie* case of obviousness has not been established because (1) Lishanski, Lau and Spivack, taken together or separately, fail to teach every limitation of the claimed invention; (2) there is no motivation or expectation of success to combine the references to arrive at the claimed invention because Spivack teaches directly away from the combination; and (3) the hypothetical combination proposed by the Examiner does not result in the claimed invention. Accordingly, removal of this ground of rejection is respectfully requested.

Thus, as with independent Claim 21, dependent Claims 23 and 24 are not obvious over the cited art. Applicant respectfully requests withdrawal of this ground of rejection.

Conclusion

In view of the foregoing amendments and remarks, applicant submits that all pending claims are in condition for allowance. Reconsideration and favorable action are requested. If any issues remain that may be expeditiously addressed in a telephone interview, the Examiner is encouraged to telephone applicant's attorney at 206.695.1655.

Respectfully submitted,

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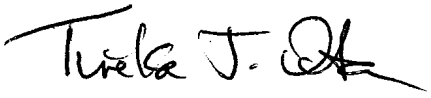

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Exhibit I - Target-specific primer sequences incorporated into the forward and reverse primers used to detect single nucleotide polymorphisms in human genomic DNA, as described in Lishanski A., Screening for Single-Nucleotide Polymorphisms Using Branch Migration Inhibition in PCR--amplified DNA," *Clinical Chemistry* 46:9, 1464-1470 (2000). Target-specific sequences were obtained from the NCBI SNP database, as described in Lishanski, pg 1468.

Submitter SNP ID #	**Reference SNP ID #**	**Primer A Target-Specific Sequence**	**Primer A Length (nucleotides)**	**Primer B Target-Specific Sequence**	**Primer B Length (nucleotides)**
ss3989	rs3951	TGAGAGTAGCTTGGCTGGGT	20	TTTGGCTTTCATCTTCCCC	19
ss4141	rs4101	ACCACATCCTCTCATTCGTTG	21	GGGGTCTCTGCAGTTAACCA	20
ss4212	rs4170	TGATGTCAAAATAGCTCCATGC	22	AATATGCAAAGTAATTTTCTGGCC	24
ss4213	rs4171	AAAACCCTGTTGATATTGGCC	21	CTGAATACTCTCCATCCTTGCC	22
ss4214	rs4172	TCGGCAGAATATACTGCATCC	21	GATGTTAGGGAAGCAAAATTGC	22
ss4215	rs4173	CTGTGTTATTTGCTGATCCTG	21	GTAAACTTTCTGAGCCTCTGG	21
ss4216	rs4174	GCCATTGTAAGATCTGAATGAGG	23	ATGTTTTATGTGGAGAGGTATCTGC	25
ss4030	rs3991	TTAATGCAGTACATGTCCTTTTGG	24	CAAGAGTTCTTGGGGGCATA	20
ss4031	rs3992	GATCGCTGTTTTTGAGCCTC	20	GGAAGTCACTTCCCTCAATACG	22